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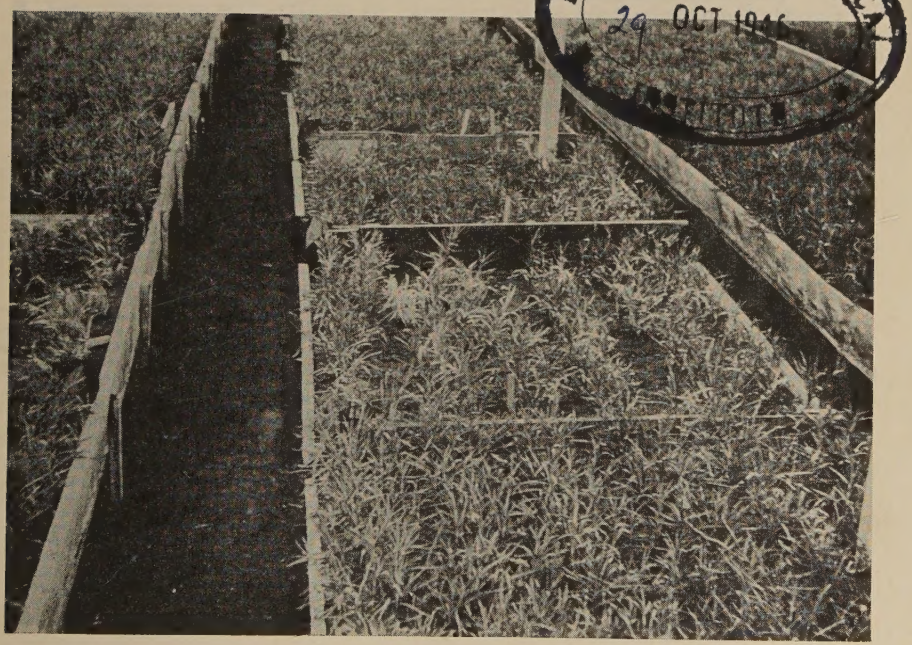
J. M. Bickerton



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FUSARIUM WILT OF CARNATIONS CAUSED BY *FUSARIUM DIANTHI* PRILL. ET DEL.[1]

J. M. BICKERTON

From 1933 to 1940 the writer made a study of diseases of greenhouse carnations (*Dianthus caryophyllus* Linn.) on Long Island. It was observed that Fusarium wilt was causing serious losses to this important florists' crop. This paper represents the results of an investigation of the life history and control of this disease under conditions on Long Island.

NAMES OF THE DISEASE

The Fusarium-wilt disease of carnations has been given such names as *stem rot, dry stem rot, die-back, crown rot, basal rot, branch rot, root rot, collar rot, consumption, straw disease,* and *Fusariose.* Most of these terms are either confusing or the symptoms implied are nonpredominant or nonexistent. The term *Fusarium wilt* proposed by Wickens (36) is in common use at present. This name is retained here even though wilting is generally an inconspicuous and late symptom of the disease, at least on Long Island. In view of the characteristic yellowing usually associated with the disease, the name *Fusarium yellows* would be more descriptive but it seems best to reserve the term *yellows* for a disease of carnations caused by a virus.

HISTORY AND RANGE

The history of the disease is obscure, particularly in early reports, because of inadequate descriptions of the symptoms and pathogen. Probably the first report in the literature of a disease resembling Fusarium wilt was made by Sturgis (28) in 1898, who observed it in Connecticut greenhouses the previous year. In 1899 the disease was recognized in New York by Stewart (26), and in the French Riviera by Prillieux and Delacroix (22) and Mangin (17). The following year Delacroix (8) published the first detailed study of Fusarium wilt and named the pathogen *Fusarium Dianthi,* n.sp. Subsequently, what appears to be the same disease has been reported in South Africa (30), Uganda (23), Denmark (13), Italy (20), Czecho-Slovakia (40), England (2), Belgium (19), Germany (38), and New Zealand (7).

The distribution of Fusarium wilt in the United States is not known with certainty. Wilt caused by *Fusarium Dianthi* or *Fusarium* sp., however, has been reported[2] from Alabama, California, Colorado, Delaware, Florida, Massachusetts, Michigan, Montana, Nebraska, New Jersey, New York,

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[1]Also presented to the Faculty of the Graduate School of Cornell University, February 1941, as a major thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

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Appreciation is also due to the New York Florists' Club for establishing the fellowship under which the work was done; to Howard H. Campbell for furnishing laboratory and office facilities in the Nassau County Farm Bureau Office; and to the various Long Island carnation growers whose cooperation and support made the field work possible.

[2]This information was kindly furnished in a letter from Dr. H. A. Edson of the Division of Mycology and Disease Survey of the United States Department of Agriculture, from a survey of the literature made by Miss Woods of his staff.

Ohio, Pennsylvania, Virginia, and Washington. In addition, the writer has identified specimens of the disease from Illinois.

ECONOMIC IMPORTANCE

According to the writer's observations, particularly on Long Island, losses caused by Fusarium wilt are probably exceeded only by those due to Alternaria blight. This, however, may be merely a matter of opinion, since Guba (14) claims that Fusarium "stem rot" or "wilt" is the most important carnation disease in Massachusetts. Although, under conditions favorable for infection, Alternaria blight attacks a majority of the carnation varieties on Long Island, Fusarium wilt appears to be confined to a few varieties. In France, Delacroix (8) and Fron (11) claim that the future of the great carnation industry in the French Riviera has been threatened by this disease. In England, Wickens (36) and White (34) (35) are agreed that Fusarium wilt is much less important than Verticillium wilt, and occurs rather infrequently.

Unlike Alternaria blight, from which plants usually recover, Fusarium wilt generally kills the affected plant. Once established in field or greenhouse, the disease may cause a complete loss of certain varieties. Plants in all stages of growth may be affected. Infection is often observed to some extent in young plants before they are transplanted to the field. Additional losses occur in the field. In one field in 1937, a loss of 60 per cent of the variety My Love was recorded, but this is rather unusual. Fusarium wilt differs in another important respect from Alternaria blight: the disease is often serious on plants grown in the greenhouse during the summer.

Losses from the disease in the field or greenhouse prior to the final benching are of slight importance in comparison to subsequent losses. In the permanent location in the greenhouse, most infection occurs in the fall and spring, and decreases to some extent during the winter. Large areas or frequently entire benches of very susceptible varieties may be killed before Christmas, causing extremely large monetary losses.

VARIETAL SUSCEPTIBILITY

Various authors have noted apparent differences in the susceptibility of carnation varieties to Fusarium wilt (4) (5) (8) (11) (21) (28) (38). Systematic observations made on Long Island have shown consistent differences in this respect among commercial varieties. Certain varieties, such as My Love and Spectrum Supreme, have been almost discarded because of their extreme susceptibility to the disease. On the other hand, many varieties, such as Puritan, Patrician, Peter Fisher, and Maine Sunshine, appear to be highly resistant. Inoculations of ten varieties with five isolates of the pathogen have confirmed field observations (table 2).

SYMPTOMATOLOGY

MORPHOLOGIC SYMPTOMS

An examination of the literature clearly shows that Fusarium wilt, caused by *Fusarium Dianthi,* has been confused with and inadequately differentiated from several other diseases of carnations. This confusion is to some extent indicated in the various names which apparently have been given the disease (page 3). The work of Dowson (10), Wickens (36), and

TABLE 1. SUMMARY OF RESULTS OF INOCULATIONS OF PLANTS OF THE VARIETIES MY LOVE, CHIEF KOKOMO, AND MATCHLESS WITH TWO ISOLATES OF *Fusarium Dianthi*

Temperature maintained (degrees Fahrenheit)	Treatment	Number of trials	Total number plants inoculated	Total number developing infection	Range of incubation period (in days)
70–80	A. Inoculated in wound at base of stem Checks: wounded at base of stem, not inoculated	2	16 10	14 0	12–28
	B. Roots dipped in water suspension of inoculum Checks: dipped in sterile water	4	103 45	95 0	23–45
60–70	A. Inoculated in wound at base of stem Checks: wounded at base of stem, not inoculated	1	8 4	8 0	40
	B. Roots dipped in water suspension of inoculum Checks: dipped in sterile water	1	36 18	24 0	50–70
50–65	A. Inoculated in wound at base of stem Checks: wounded at base of stem, not inoculated	1	8 4	7 0	41
	B. Roots dipped in water suspension of inoculum Checks: dipped in sterile water	2	76 38	30 0	56–75

TABLE 2. RESULTS OF INOCULATIONS OF TEN VARIETIES OF CARNATIONS WITH FIVE ISOLATES OF *Fusarium Dianthi*

Variety	Per cent infection 3 months after inoculation; and sources of isolate						Degree of susceptibility as observed in the field 1937–1940
	C7 Long Island	C5 Long Island	C22 Long Island Overwintered	C18 Illinois	C23 Massachusetts	Checks not inoculated	
1. My Love	100	80	100	100	90	0	Very susceptible
2. Spectrum Supreme	100	0	70	100	30	0	Very susceptible
3. Chief Kokomo	70	0	0	30	80	0	Very susceptible
4. Sophelia	10	0	60	0	20	0	Moderately susceptible
5. Virginia	90	70	20	0	40	0	Moderately susceptible
6. Puritan	0	0	0	10	0	0	Very slightly susceptible
7. Peter Fisher	0	0	0	0	0	0	Very slightly susceptible
8. King Cardinal	0	0	0	0	0	0	Slightly susceptible
9. Patrician	0	0	0	0	0	0	Very slightly susceptible
10. Maine Sunshine	0	0	0	0	0	0	Very slightly susceptible

White (33) (34) (35) in England is of inestimable importance in clarifying this disease complex. Wickens (36) and White (35) present information of diagnostic value in separating Fusarium wilt from stem rot caused by various Fusarium species and Verticillium wilt. The following presentation of progressive symptoms of Fusarium wilt is based on numerous observations of various stages of the disease in artificially and naturally inoculated plants.

On the leaves and stems

A characteristic feature of Fusarium wilt infection is the unilateral development of symptoms on the tops (figures 1 and 2). The first outward symptom appears at the base of the stem on the affected side as a browning or yellowing of the stem internodes, a yellowing, followed by withering, of the leaf bases and a yellowing of midveins of these leaves to the tip. Grad-

ually the infected leaves become yellow, often on one side at first, and finally wither. These symptoms slowly progress up one side of the plant and into the lateral branches and shoots.

FIGURE 1. FUSARIUM WILT OF A YOUNG, NATURALLY INOCULATED PLANT OF THE VARIETY SOPHELIA

Note the yellowing and withering of leaves on the affected side and the bending of the terminal leaves.

As infection nears the end of a shoot, the terminal leaves arising on the infected side at first become chlorotic and curl outward (figures 1, 2, 3, 5). The chlorosis or yellowing, which is often initially one-sided, and the curling of these leaves become more and more accentuated, giving a characteristic

FIGURE 2. FUSARIUM WILT OF PLANTS OF THE VARIETY MATCHLESS, 60 DAYS AFTER INOCULATION IN A WOUND AT THE BASE OF THE STEM

appearance. At this stage vertical cracks sometimes appear in the withered stem in the internodes. On some red varieties, such as Carlotta, the affected leaves exhibit a purplish rather than a yellowish color. The one-sided effect on shoots may also occur on the flower stems with a consequent crippling of the flowers.

Stunting of shoots or entire plants may occur, especially if infection is initiated early in the life of the plant. Following the initial one-sided development in the main stem and shoots, the entire plant finally becomes infected as a result of lateral growth of the pathogen. Long after the death of most of the plants, a few weak green shoots may still be evident. Finally, however, such shoots, which sometimes develop from adventitious buds in the lower part of the main stem, also become withered and straw-colored.

Within affected stems

The outward symptoms on an affected plant develop in accordance with the upward growth of the pathogen in the vascular system on one side of the stem. This is made evident by the upward extension of vascular discoloration on the affected side (figure 3). The discoloration gradually extends laterally in the affected stems into the pith and cortex, where a brown soft rot develops. The affected tissue is sharply demarcated from neighboring healthy tissue.

The brown discoloration in the stem tissue can be clearly observed by "pinching out" an infected shoot. As the disorganization continues, the internal tissue finally becomes shredded. At this stage the stem feels hollow to the touch and shoots can be pulled out at the internodes with very slight effort. In established plants, the brown discoloration of the pith and cortex in the lower part of the main stem may become extensive; but a wet rot of this part of the stem is in most cases not evident until advanced stages of infection, when almost the entire plant has been killed. After large numbers of young rooted plants had been inoculated with the pathogen, a rapid soft rot of the basal part of the stem and a rapid wilting of the foliage developed in a very small percentage of cases.

On the roots

Extensive rotting of the roots does not generally occur until very late stages of the disease. Because infection in most cases appears to be initiated in the roots, one rotted root, or a few such roots, can be observed, usually on one side of the plant, in early stages of the disease. Vascular discoloration in these roots extends upward into the stem.

SIGNS

Signs of Fusarium wilt are the pink to orange-colored clusters of spore-bearing sporodochia and pionnotes; the minute, white to buff-colored sclerotial bodies; and the white mycelium of the pathogen. These structures are found most frequently near the base of the main stem, in the joints of branches, or on the stem in the leaf axils.

ETIOLOGY

Fusarium wilt of carnations is caused by *Fusarium Dianthi* Prillieux and Delacroix. Wollenweber and Reinking (39) class this fungus in the subgroup Oxysporum of the section Elegans. Snyder and Hansen (25) propose to change this name to *F. oxysporum f. Dianthi* (Prill. et Del.)

FIGURE 3. LONGITUDINAL SECTION THROUGH A STEM OF A PLANT AFFECTED WITH FUSARIUM WILT SHOWING TYPICAL UNILATERAL, BROWN, VASCULAR DISCOLORATION

n. comb.; but because this has not been generally accepted at the present time, the writer proposes to follow Wollenweber and Reinking (39) in using the original name.

Sturgis (28) in 1897 appears to have been the first to isolate a species of Fusarium from carnations affected with a disease resembling Fusarium wilt. In 1899 Prillieux and Delacroix (22) isolated the pathogen and gave it the present name. Later, Wight (37) and Van der Bijl (30) briefly described unnamed species of Fusarium causing a stem rot and a wilt, respectively, of carnations. In 1922, Small (24) claimed that a wilt of carnations and several other plants in Uganda was due to a species of Fusarium identified as *F. udum* Butler. He noted its resemblance to *F. Dianthi* but claimed certain minor differences. Seven years later White (33) and Dowson (10), working independently in England, published the results of their study of stem rot and wilt diseases of carnations. They concluded that several species of Fusarium, particularly *F. culmorum* (W.G.S.) Sacc., were involved in this complex, but made no mention of *F. Dianthi*. It remained for Wickens (36) in 1935 to show that Fusarium wilt caused by *F. Dianthi* is distinct from the stem rot and wilt diseases caused by other Fusarium species and *Verticillium cinerescens*.

Numerous isolates of Fusarium were readily obtained from the stems and roots of carnations affected with Fusarium wilt. The majority of the isolates from Long Island material, several of which were tested for pathogenicity with positive results, appeared to be more or less morphologically alike. Single-spore cultures were made of several of these, and, on the basis of macroscopic appearance in culture, the original isolates were separated into two groups. Two single-spore isolates, one typical of each group, were used in subsequent identification and inoculation work. In addition, one single-spore isolate from Massachusetts and one from Illinois, obtained from infected plants, were used. These four isolates were grown in tube cultures of various media, as recommended by Dr. O. A. Reinking of the New York State (Geneva) Experiment Station. These media included 1.5 per cent potato-dextrose agar, boiled rice, elm twigs, and wheat heads. The cultures were incubated for approximately two weeks in subdued light and an additional two or three weeks in sunlight, and were then examined for macroscopic and microscopic characters. The results were compared with the descriptions given by Wollenweber and Reinking (39).

The two Long Island isolates were definitely identified by Dr. W. C. Snyder of the University of California and Dr. O. A. Reinking as belonging to the section Elegans, and tentatively identified by both authorities as being *Fusarium Dianthi*. This confirms the writer's observations that, on the whole, the four isolates agree with the description of *F. Dianthi* in regard to sporodochial and sclerotial characters, the production of terminal and intercalary chlamydospores, and the size, shape, and number of septa of microconidia and macroconidia. Slight differences which were observed in regard to color characteristics and spore size are not considered significant. Illustrations of the essential spore characters of these isolates are shown in figure 4. The pathogenicity of the isolates has been established as described below. Indications of pathologic strains of *F. Dianthi* are shown in the results (table 2) of inoculations of ten varieties of carnations with five isolates of the pathogen, although the evidence is by no means conclusive.

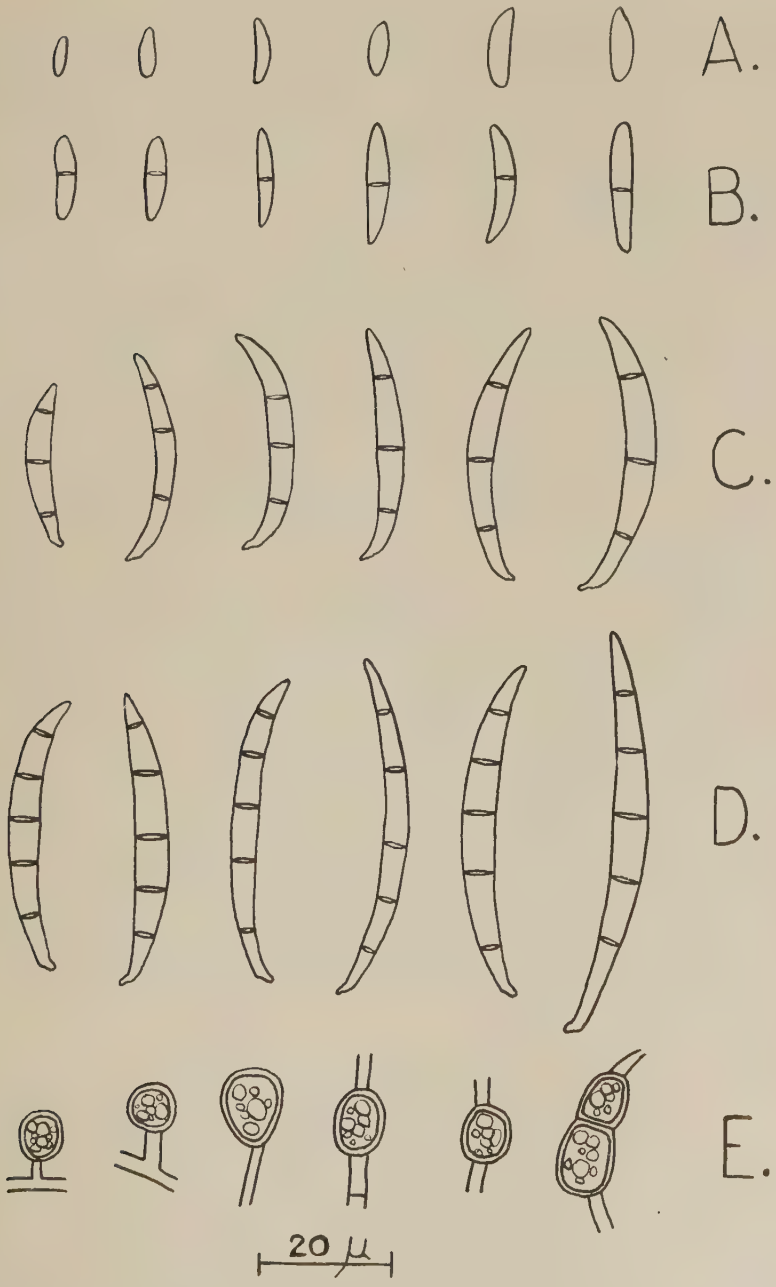

FIGURE 4. (A-D) SPORES FROM VARIOUS ISOLATES OF *F. Dianthi* SHOWING VARIATION IN THE SHAPE, SIZE, AND SEPTATION OF CONIDIA. (E) TERMINAL AND INTERCALARY CHLAMYDOSPORES

Drawings made with the aid of a camera lucida.

PATHOGENICITY

Inoculations with Fusarium isolates were attempted by several early workers (6) (8) (18) (23) (28) (30) (37), but the results are inconclusive, owing in some instances to uncertainty as to the organism involved, or to failure to apply the rules of proof of pathogenicity. Wickens (36) and White (35) describe successful inoculations with, and re-isolations of, pure cultures of *F. Dianthi.*

Numerous inoculations were made by the author with single-spore isolates of the pathogen, which were readily obtained, using the method described by Kienholz (16). The results of inoculations with two Long Island isolates are summarized in table 1. Rooted plants of the varieties Matchless, My Love, Sophelia, and Chief Kokomo, which varied in age from one to six months, were used in these tests. After inoculation, the plants were kept in the greenhouse at various temperatures, as shown in table 1.

In some cases inoculations were made by inserting the inoculum into a wound near the base of the stem. Because of the inconsistent results which were obtained by growing plants in artificially infested soil, other root inoculations were made, using the method proposed by Wellman (32) with slight modifications. Both methods resulted in typical infection in most cases (figures 2 and 5), and successful re-isolations of the pathogen were made from infected plants.

To test varietal susceptibility to Fusarium wilt, inoculations by means of the Wellman method were made July 16, 1940, with five single-spore isolates of *F. Dianthi* on ten varieties of carnations. The inoculated plants were grown in flats of disinfested soil in the field. In addition to the two Long Island isolates employed in the above experiments, a third isolate obtained from infected plants buried in the field during the winter of 1939–40 was used in this test. One isolate from Illinois and one from Massachusetts were also used. Ten plants of each variety were inoculated with each isolate. Each lot of ten plants was planted in a separate flat. Other flats of each variety containing a like number of plants served as controls. On October 13, 1940, all plants were removed and examined for evidence of infection. The number of plants which developed infection (table 2) varied according to the variety and the isolate, but the check plants remained healthy in all cases. Re-isolations were attempted from some of the infected plants inoculated with the Illinois, Massachusetts, and overwintered Long Island isolates. The pathogen was successfully recovered in each instance.

LIFE HISTORY OF THE PATHOGEN

SOURCES OF INOCULUM

Infected cuttings

As early as 1899 Mangin (17) claimed that the pathogen, presumably *F. Dianthi*, carries over in cuttings from infected plants. As evidence he stated that isolated infected plants appeared among healthy plants; the disease often appeared on new soils; and mycelium was observed in apparently healthy stems. Similar claims have been made by several other authors (1) (3) (5) (8) (14) (20) (38), but final proof is lacking.

Owing to the progressive nature of Fusarium wilt infection, various numbers of green shoots are often evident on infected plants. Such plants

frequently appear among healthy plants in the benches, and thus it seems likely that healthy-appearing cuttings from infected plants may be taken along with those from healthy plants. Several experiments were conducted to determine whether the pathogen is transported in such cuttings.

In one experiment, top cuttings of a number of varieties were taken as follows: (a) 50 green cuttings from healthy plants; (b) 50 green cuttings from infected plants; and (c) 50 yellow cuttings from infected plants. These were "stuck" in new sand in a propagating house, with wide spaces between the lots. All of the yellow cuttings failed to root and finally withered. At the end of four weeks, 90 per cent of the green cuttings from healthy plants had rooted, whereas only approximately 30 per cent from infected plants had formed roots, the remainder having withered.

In an effort to determine the extent of the longitudinal invasion of the pathogen in a given branch, serial isolations were attempted, from vascular tissue in the internodes, by starting at the tip and progressing toward the base of the branch. It was found that the fungus is always present where vascular discoloration is evident, and is sometimes present where no vascular discoloration is visible.

In other experiments, isolations were attempted from the vascular tissue of cuttings selected from infected plants and neighboring healthy plants in various greenhouses. The results of these experiments are summarized in table 3. These data show that the pathogen never occurs in cuttings from apparently healthy plants, but is always present in yellow cuttings from infected plants. The important fact is, however, that it is frequently present in green shoots on infected plants, particularly where vascular discoloration is evident.

From the above evidence, it appears likely that *Fusarium Dianthi* may carry over in some cuttings which are thus infected when inserted in the propagating sand. Probably the mycelium grows out of an unrooted, infected cutting and infests the sand; or, if the cutting roots, the hyphae may later grow from the rotting root and stem tissue into the surrounding soil. Thus, such cuttings may serve as a source of inoculum for primary and secondary infections, as observations in commercial greenhouses indicate.

TABLE 3. ISOLATION OF *Fusarium Dianthi* FROM VARIOUS TYPES OF CARNATION CUTTINGS

Type of cutting	Number of cuttings taken	Number of tissue plantings made	Number of plantings yielding Fusarium
Green cuttings, healthy plants	28	92	0
Green cuttings, no vascular discoloration, infected plants	20	76	12
Green cuttings, vascular discoloration, infected plants	30	96	53
Yellow cuttings, vascular discoloration, infected plants	30	96	95

Infected plants

Numerous observations have shown that very little infection appears in the young stock prior to transplanting, doubtless owing to the prevailing low temperatures at this time. Toward the end of April, however, as high as two-per-cent infection has been observed in the young plants of a very

susceptible variety grown in soil known to be infested with the pathogen. These plants are often not discarded by the grower when transplanting to the field. In addition, in experiments for the control of this disease, a few scattered infected plants have become evident in virgin land one to four weeks after transplanting, even though obviously infected plants were carefully discarded at the time of transplanting. Furthermore, the difficulty of excluding plants showing early symptoms of the disease and those in a latent period of infection, at the time of transplanting from the field to the greenhouse, has been repeatedly demonstrated in control experiments. Consequently, the transfer of infected plants to the field and greenhouse may provide a source of inoculum for secondary infections.

Infested sand in propagating benches

The new-washed sand which is used in a majority of the propagating benches on Long Island is presumably not infested with *F. Dianthi;* it may become infested, however, by the introduction of infected cuttings as described above. Likewise, as pointed out by Guba (14), the cuttings may be infested with spores of the pathogen which could serve to infest the sand.

Soil in the greenhouse and field

The soil available to many Long Island carnation growers is undoubtedly heavily infested with the pathogen, as shown by the annual losses from Fusarium wilt of very susceptible varieties in the field and greenhouse. Like other pathogenic species of this group, *F. Dianthi* probably persists for long periods, once established in a certain soil. Delacroix (9) believed that the thick-walled chlamydospores, formed by the fungus in the decaying tissue of infected plants and the surrounding soil, have the ability to survive for at least eleven months under adverse environmental conditions.

In experiments to determine the ability of *F. Dianthi* to survive under field conditions on Long Island three methods were used: (a) One-quart milk bottles containing pure cultures of a pathogenic isolate growing in sand-wheat-water medium were buried six inches below the soil surface; (b) similar cultures of this isolate were grown in vials one-half inch in length and diameter; these were attached at intervals to strips of cardboard that were buried vertically in the field so that the uppermost vials were at surface level of the soil and the lowest were 18 inches below the surface; and, (c) several infected plants of the variety Sophelia were placed in the field in a flat covered with soil.

All materials were buried in the fall. The following spring attempts were made to recover the fungus in plates of sterile agar. Pure cultures of the pathogen were readily obtained in most cases. In the case of the bottles this was accomplished after they were buried two successive winters in the field, the second winter (1939–40) being exceptionally severe. The fungus was recovered from the vials at all levels in the soil after one winter. The results of inoculations of several varieties of carnations with an isolate (C22) obtained from the overwintered plants are shown in table 2. Successful re-isolations of the pathogen were made from the infected plants.

Microscopic examinations of the overwintered inoculum in the bottles, vials, and infected plants revealed the presence of numerous hyphae and chlamydospores. Several attempts to germinate chlamydospores obtained

from the infected plants, in distilled or tap water at room temperatures, were unsuccessful after 20 days. The hyphae made considerable growth under these conditions, however.

From the above experiments it is evident that, under conditions on Long Island, *F. Dianthi* is able to persist in the soil at least one winter and probably longer. When in contact with rotting carnation tissue, the fungus is strongly virulent in the spring, after one winter in the field. Thus, carnation debris which is left in the field in the summer doubtless provides a source of inoculum for infections in the field the following year.

INFECTION COURTS

Mangin (18), Delacroix (8), Casse (5) in 1900, and Wight (37) in 1912 claimed, but did not fully substantiate, that infection occurs largely through wounds in the stem. In 1916 a small number of inoculations made by Van der Bijl (30) with an undetermined Fusarium isolate through wounds in the stem and through roots were apparently successful. Wickens (36) in 1935 made successful inoculations with *F. Dianthi* through the cut bases of cuttings and through the roots of a few established plants grown in infested soil. He was unable to produce infection by placing inoculum in the leaf axils of cuttings or nodal wounds of established plants.

The examination of large numbers of naturally infected plants has indicated that occasional infections occur through wounds in the main stem, but that the large majority are initiated in the roots. Records were taken on infected plants examined during a four-week period in 1937. Of a total of 150 plants thus inspected, 145 showed vascular discoloration extending from the roots upwards, while only 5 showed the upward extension of vascular discoloration starting from a wound in the stem.

Numerous inoculations described above (page 12) have demonstrated that infection readily occurs through roots or wounds in the stem. Attempts to obtain infection through uninjured green or woody stems were unsuccessful. Because no precautions were taken in root inoculations to prevent root injury, it is not known whether infection will occur through uninjured roots. This is of slight importance, however, because injured roots are probably always present under natural conditions.

INCUBATION

In the various inoculations made with *F. Dianthi*, careful records were kept of the period from inoculation until the appearance of initial symptoms of the disease. The length of this period varies according to the prevailing temperatures and the method of inoculation (table 1). At 70–80° F. the incubation period ranged from 12–28 days in the case of stem-wound inoculations to 23–45 days with root inoculations. At 50–65° F., however, this period lengthened to 41 days in the case of stem-wound inoculations and 56–75 days in the case of root inoculations. In additional root inoculations in the field to test varietal susceptibility (table 2), the incubation period extended from approximately 35 to 60 days.

Wickens (36) stated that the incubation period varied from 49–70 days in the case of nodal wound inoculations on rooted cuttings to 98 days on plants grown in infested soil. White (34) reported that infection appeared in about 75 days but did not describe the method of inoculation. The tem-

peratures at which the inoculated plants were held were not given by either author. Their results, however, agree with those of the writer in demonstrating that the latent period of infection may be of several weeks' duration. The significance of this long period, discussed on page 14 is in the increased difficulty of excluding infected plants from the field and greenhouse.

INFECTION

Prillieux and Delacroix (22) in 1899 described the growth of the mycelium of the pathogen up the stem through the vessels and the gradual lateral penetration of the surrounding tissues. Histological studies made by the author have confirmed their work. On entering the roots or stem, usually on one side of the plant, the hyphae extend upwards in the vessels and tracheids. A brown gum-like substance often appears in these tissues as a result of infection (36). Following the vertical extension in the xylem, the fungus slowly penetrates laterally into the pith on one side, and the cambium, phloem, and cortex on the other side. These tissues in the affected roots, main stem, and side shoots are gradually rotted and disorganized until only the vessels and tracheids remain intact. This accounts for the hollow feel and shreddy texture of stems in advanced stages of infection. Gradually the mycelium extends from one shoot to another throughout the plant until the entire plant is killed. Terminal and intercalary chlamydospores are formed in abundance by the ramifying mycelium within the affected tissues.

EPIPHYTOLOGY

EFFECT OF TEMPERATURE

On mycelial growth

Wickens (36) reported that the optimum temperature for the growth of *Fusarium Dianthi* in culture is 84.2° F. He claimed, further, that the fungus grows strongly at 95° F., but not at all at 50° F. Three trials similarly conducted by the author, with two isolates of the pathogen growing on potato dextrose agar at temperatures ranging from 35° to 100° F., agree in showing that the optimum, minimum, and maximum temperatures for the growth of the pathogen are approximately 80°, 43°, and 97° F., respectively.

On infection

Stone (27) in 1910 made the observation that the amount of "dry stem rot" of carnations caused by a species of Fusarium (presumably *F. Dianthi*), is increased by extensive forcing of carnations at high temperatures and decreased by low temperatures. Guba (14) in 1931 made similar observations in regard to "stem rot" or "wilt," likewise caused by an unidentified species of Fusarium. The effect of temperature on Fusarium wilt has been strikingly demonstrated in most of the inoculations made in the present investigation (figure 5).

Table 1 shows that the amount of infection that developed following stem-wound inoculations was little influenced by the prevailing temperature, presumably because the inoculum was introduced directly into the stem. On the other hand, in the case of root inoculations, the amount of infection was much greater at higher than at lower temperatures. For

example, of the total plants inoculated at each temperature, 92 per cent became infected at 70–80° F., 66 per cent at 60–70° F., and only 39 per cent at 50–65° F. Furthermore, in the case of both stem-wound and root inoculations, the length of the incubation period was decreased as the temperature increased.

FIGURE 5. EFFECT OF AIR TEMPERATURES ON THE AMOUNT OF FUSARIUM WILT IN PLANTS OF THE VARIETY SOPHELIA DURING A PERIOD OF 38 DAYS AFTER ROOT INOCULATIONS WERE MADE

(1) At 70–75° F., abundant infection
(2) At 60–65° F., no infection
(3) At 50–55° F., no infection

In regard to longevity, it was observed in the above inoculations that at 75–80° F. the infected plants withered and died soon after infection appeared; on the other hand, many of those at the lower temperatures were still growing actively three months after they became infected. These observations agree with those made in commercial greenhouses. Under natural conditions the greatest amount of infection occurs during the summer, early fall, and spring, with a decrease during the winter. Plants developing infection in October and November, when the prevailing greenhouse temperatures are comparatively low, have been observed to continue growth and to produce flowers for several weeks before dying.

CONTROL

SELECTION OF CUTTINGS

The danger of transmitting the pathogen of Fusarium wilt in healthy appearing cuttings from infected plants is discussed above (pages 12–13). In view of this possibility, it might be advisable to remove and destroy infected plants as soon as they become evident in the benches. On the other hand, as such plants may continue to produce flowers for some time during

the winter, their destruction may be unwarranted. It may be more practical to avoid taking cuttings from the neighborhood of infected plants. An investigator in Colorado (1) claims that the practice of taking cuttings from disease-free stock, together with ordinary sanitary measures, has made it possible to control the disease.

CONTROLLING AIR TEMPERATURES

Although the amount of Fusarium-wilt infection is decreased at low temperatures (table 1), it is doubtful if this knowledge can be advantageously used for controlling the disease in commercial greenhouses. To maintain air temperatures low enough in the summer, early fall, and late spring, when the greatest losses from the disease occur, is apparently impossible. Since carnations grow best around 50° F., a majority of the growers make a practice, as soon as possible in the fall, of maintaining night temperatures near this point. The effect of reducing greenhouse temperatures by increased ventilation and prolonged shading after benching from the field has not been investigated, but appears to be of doubtful value as a control factor.

ROTATION OF FIELD SOIL AND GREENHOUSE-BENCH SOIL

Indirect evidence in favor of rotation of field soil for Fusarium-wilt

FIGURE 6. EFFECT OF OLD AND NEW FIELD SOIL ON AMOUNT OF FUSARIUM WILT IN THE VARIETY MY LOVE

The plots of diseased plants were grown prior to benching in old carnation soil. The plots of healthy plants were grown in new soil prior to benching. Photograph was taken 4 weeks after benching, 1940.

control was obtained in experiments on soil disinfestation. In one experiment, conducted in 1939, approximately 900 plants of the variety Sophelia

were grown in replicated plots, treated and not treated with chloropicrin, on land new to carnations; a like number were grown in similar plots on old, infested soil. At the end of August a total of 13 infected plants had appeared in the new soil plots, whereas 63 were recorded in the old soil plots. The results of a similar experiment conducted with the variety My Love in 1940 (table 4) show that almost nine times as much infection developed on old land as on new land (figure 6). The difference was highly significant.[3] Because the young plants used in both these experiments were previously grown in old soil in the greenhouse, the appearance of infection in new soil can be explained on the basis of the carry-over of plants in a latent period of infection and of those with infested soil on the roots. Although final data in both experiments were taken toward the end of August a few weeks after benching, the long incubation period leads one to believe that all infections recorded after benching were initiated in the field.

Although the above evidence indicates the probable advantage of rotation in controlling Fusarium wilt, the period required for the pathogen to die out in a given soil in the absence of carnations is not known with certainty. Delacroix (8) asserted that a three-year rotation should be adequate, since chlamydospores held dry in culture did not survive longer than a year. Evidence presented on pages 14–15 show that on Long Island the pathogen is able to survive at least one winter and probably two winters. Consequently, it is advisable to suggest only that carnations be grown on a particular unit of land no more often than necessary, having in mind both the field and greenhouse soil. In establishing such a rotation consideration should be given to such sanitary measures as (a) the proper disposal in the field of the old bench soil, and (b) the accumulation and destruction of plant debris from the field and greenhouse rather than strewing it in the field.

TABLE 4. A COMPARISON OF THE AMOUNT OF FUSARIUM WILT ON PLANTS OF THE VARIETY MY LOVE GROWN IN OLD AND NEW FIELD SOIL, TREATED AND NOT TREATED WITH CHLOROPICRIN IN EACH CASE, 1940

	Average number of infected plants counted up to August 20		
	Grown in old land in field	Grown in new land in field	Mean*
Treated with chloropicrin	11.50	5.12	8.31
Not treated with chloropicrin	61.87	3.37	32.62
Mean*	36.69	4.25	

*Difference necessary for odds 19:1 = 4.38 plants.
Difference necessary for odds 99:1 = 5.91 plants.

SELECTION OF RESISTANT VARIETIES

The results (table 2) of inoculations of several commercial varieties of carnations indicate that many of these are truly resistant to *F. Dianthi.* Therefore, such varieties should be grown in preference to the more susceptible kinds. The cultivation of many of the latter varieties appears to be necessary, however, at the present time, to meet the large demands of

[3]All statistical treatments of data in this text were made by the analysis-of-variance method

the retail trade in regard to color and other desired characteristics. But it would seem that many new varieties possessing the desired qualities could be developed from the resistant types.

SOIL DISINFESTATION

Many workers have attempted the control of Fusarium wilt by means of soil disinfestation, but the results were apparently negative or the evidence was inconclusive (8) (11) (12) (15) (27) (30). In discussing the potentialities of soil disinfestation for this purpose, Guba (15) states that "unless we can improve our methods of propagating and growing previous to benching, there is not much to be said in favor of bench soil sterilization".

Several experiments were conducted on Long Island in an attempt to control Fusarium wilt by means of soil disinfestation. Owing to the apparent danger of re-infesting treated bench soil by transplanting infected plants and infested soil from the field, disinfestation of both field and bench soil was assumed necessary. Steam sterilization appeared impractical because the majority of Long Island growers are not equipped to use steam, particularly for field work. The standard method of using an aqueous formaldehyde solution seemed possible for bench treatment, but impractical for field treatment because of the necessity of hauling large quantities of water to the field. One solution of this difficulty appeared to be the injection of concentrated forty-per-cent commercial formalin directly into the field soil. The use of chloropicrin, a promising soil disinfestant, also seemed worthy of trial.

Two experiments, the first in 1938 with both formaldehyde and chloropicrin, and the second in 1939 with chloropicrin alone, were conducted. Both experiments were conducted on naturally infested soil (unless otherwise stated), and the moderately susceptible variety Sophelia was used. In addition, precautions to reduce re-infestation of the treated plots were taken in these and subsequent experiments. In all experiments the holes were filled in with soil immediately after injection of the fungicides, and the treated soil was later turned over once or twice to facilitate rapid removal of the vapors.

In the 1938 experiment a bed in the field was divided into twenty plots each having an area of 105 square feet. Four replications of the following five treatments were randomized in this bed:

Treatment 1. Plants set in untreated soil.

Treatment 2. Plants set in untreated soil; later planted in the greenhouse in soil treated with chloropicrin.

Treatment 3. Plants set in soil treated with concentrated commercial formalin, applied April 19–25 at the rate of one gallon to fifty square feet of soil by pouring to a depth of six inches in holes spaced one to every four square feet.

Treatment 4. Plants set in soil treated with concentrated commercial formalin, similarly applied April 19–25 at the rate of one gallon to fifty square feet in six-inch holes spaced one in every square foot.

Treatment 5. Plants set in soil treated with chloropicrin, applied April 21–22 by injection at the rate of two c.c. per square foot in holes six inches in depth, spaced at one-foot intervals.

Four days after the material was applied, no indication of chloropicrin

vapor could be detected. On the other hand, even though vigorously stirred and washed with rain, the soil treated with formalin retained detectable fumes as long as 37 to 43 days after application, resulting in considerable injury to plants set during this period. All plots were planted May 6–8. Less than two-per-cent Fusarium-wilt infection appeared in any of the plots in the field.

On June 27 a bench in the greenhouse containing old infested soil was divided into twenty plots, each having an area of approximately 25 square feet. This division gave four replications of each of the following five treatments randomized in the bench:

Treatment 1. Check, untreated, planted July 12 with field plants grown in untreated soil in the field (field treatment 1 above).

Treatment 2. Plants grown in untreated soil in the field (field treatment 1 above); field soil washed from roots with water and planted July 12 in untreated bench soil. This treatment, which proved of no value, was included because of promising results obtained the previous season.

Treatment 3. The bench soil was treated June 30 with chloropicrin at the rate of two c.c. per square foot, inserted in holes, four inches in depth, spaced one foot apart; covered tightly with black building-paper for three days. These plots were planted July 12 with plants grown in untreated soil in the field (field treatment 2 above).

Treatment 4. The bench soil was treated June 28 with a 1–25 aqueous solution of formaldehyde applied at the rate of one-half gallon to one square foot. This resulted in the application of one gallon of concentrated formalin to fifty square feet as used in the field. These plots were tightly covered with black building-paper for two days and planted July 12 with plants grown in field soil treated with formalin (field treatments 3 and 4 above).

Treatment 5. The bench soil was treated June 30 with chloropicrin as in treatment 3, and covered with black building-paper for three days. On July 12 these plots were set with plants grown in soil treated with chloropicrin in the field (field treatment 5 above).

Approximately 92 plants were set in each plot. Although very slight infection had developed by the middle of September, 1938, there appeared to be almost as much in the treated as in the untreated plots. Because in many of these plants infection became evident a few weeks after benching, however, it is assumed they were in a latent period of infection when transplanted from the field. By April, 1939, a majority of the plants in the treated plots were still green and apparently healthy, whereas most of the plants in the untreated plots were dead and withered. Many of the plants in the treated plots developed symptoms of the disease and died, from April 15 to June 15. Nevertheless, most of these plants had been healthy and productive throughout the winter, whereas the majority of those in the untreated plots were killed during the fall of 1938.

Flower production records were kept on the above plots from September 20, 1938, to March 25, 1939, and the average number of flowers produced per square foot of bench was calculated for each treatment. It is evident (table 5) that treating infested soil with either chloropicrin or formaldehyde doubled the yield, in comparison with the check, mostly because of a reduction in the severity of the disease. This difference in production, which is highly significant, is probably due in part to an increase in vigor

of plant growth, which was very apparent in September, 1938, from soil treatment with these fungicides. Weinard and Decker (31) note a similar effect on carnations growing in steam-sterilized soil. The difference between the chloropicrin treatment both in the field and greenhouse and the chloropicrin treatment only in the greenhouse, or between the chloropicrin and formaldehyde treatments, is not significant. The average seasonal production in the treated and untreated plots is shown graphically in figure 8.

TABLE 5. THE EFFECT, ON THE PRODUCTION OF FLOWERS OF THE VARIETY SOPHELIA, OF TREATING, WITH FORMALDEHYDE AND CHLOROPICRIN, SOIL INFESTED WITH *Fusarium Dianthi*

Treatment	Flower production per square foot of bench	
	1938–39	1939–40
	Sept. 20–Mar. 25	Sept. 1–May 1
1. Check untreated	8.35*	6.27†
2. Bench soil treated with chloropicrin	16.04	....
3. Field and bench soil treated with chloropicrin	17.81	19.92
4. Field and bench soil treated with formaldehyde	18.34	....

*Difference necessary for odds of 19:1 = 3.48
Difference necessary for odds of 99:1 = 4.87
†Difference necessary for odds of 19:1 = 2.19
Difference necessary for odds of 99:1 = 3.09

In the 1939 experiment an attempt was made to determine whether the summer location influences the subsequent progress of the disease after benching. Plants were grown (I) in the field on old soil naturally infested with the pathogen; (II) in the field in soil new to carnations; and (III) in the greenhouse during the summer in old infested soil. In each case comparisons were made on (a) untreated soil and (b) soil treated with chloropicrin both in the field or greenhouse prior to final benching and in the greenhouse at the time of benching. For the old and new field plots a wide bed was, in each case, divided into eight equal plots and, for the greenhouse plots, part of a bench was similarly divided into eight equal plots. Each alternate plot in the three locations was treated between April 25 and May 9 with chloropicrin, resulting in four replications of treated and untreated plots in each location. The treated field plots, together with the contiguous paths, were marked into one-foot squares and injections of chloropicrin, each of 1.5 c.c., were made to a depth of six inches at the rate of two injections in each square foot. These injections were uniformly staggered. This resulted in the application of three cubic centimeters per square foot.

To avoid injury to neighboring plants in the greenhouse, the soil in the summer-treated greenhouse plots was conveyed to a steel truck body, and there treated with the same rate and distribution of chloropicrin as used in the field. Subsequently this soil was returned to the greenhouse-bench plots, the boards of which had been disinfested by washing with a 1–50 formaldehyde solution.

As mentioned above, in the 1938 experiment the chloropicrin vapor rapidly passed out of the soil after treatment. In the 1939 experiment, however, even though the treated field soil was vigorously stirred twice after

treatment, the gas could still be detected 20 to 25 days after application and caused considerable injury to plants set during this period. All plots were planted by May 15 with 112 plants in each plot. Records were taken at weekly intervals of the number of infected plants which appeared in these plots.

Early in July a bench was prepared for the above plots in the greenhouse. Carnations abundantly affected with Fusarium wilt had been grown in the old soil in this bench for two successive years. After cleaning out plant debris in and around the bench and preparing the soil, the bench was divided into 24 equal plots each with an area of 24 square feet. The four replications of treated and untreated plots in each of the three summer locations were randomized in this bench.

Chloropicrin was applied July 4 to the treated bench plots with the same distribution as above at the rate of 2.5 c.c. per square foot injected in two equal parts in each square foot to a depth of four inches. The surface of the soil was kept moistened with water for three days after treatment. Each plot was planted with 80 plants on July 13.

Periodic counts were made up to September 15, 1939, of the number of plants affected with Fusarium wilt which appeared in each plot. Additional counts were made January 6, 1940, and finally July 8, 1940. From these records the total amount of infection in the treated and untreated plots was calculated for given dates throughout the season. These data, expressed as a percentage of the total number of plants, and plotted graphically (figure 7), illustrate the seasonal trend of the disease in treated and untreated soil. In addition, flower-production records were kept on all plots from September 1, 1939, to May 1, 1940. Calculations were made from these records of the average total production per square foot of bench at intervals during this period and finally, May 1, in the treated and untreated plots. The seasonal production in these plots is shown in figure 8. The final production data are presented in table 5.

Although the amount of infection which appeared up to September 15 was comparatively slight, there was considerably more on plants grown in infested soil in the field and greenhouse during the summer than on those grown in new soil. With the exception of the latter plots, however, there were just as many or more infected plants in the plots treated with chloropicrin as in those untreated, presumably caused by ineffective treatments. Subsequently, however, new infections began to appear at a rapid rate in all the untreated, infested bench plots, regardless of their summer location; whereas comparatively few new infections appeared in the treated plots. By January 6 a majority of the plants were killed in the untreated plots, and flower production had almost ceased (figures 7 and 8 respectively). However, it is evident (figure 7) that the development of infection in the treated plots occurred at a much slower rate, and by July 1, 1940, seventy per cent of these plants were still seemingly healthy. Likewise, the production of flowers in these plots increased steadily throughout the winter (figure 8).

From the data in table 5 it is apparent that more than three times as many flowers were finally produced in the treated plots as in the untreated, the difference being highly significant. These results are comparable to those obtained in the 1938 experiment (table 5). Furthermore, the seasonal

trend in production (figure 8) and infection in the 1938 experiment was very similar to that of the 1939 experiment.

Toxicity tests conducted with chloropicrin in 1939 further demonstrated the ability of this material to kill the pathogen in soil. In these tests a technique used in overwintering tests, described on page 14, was employed in which the pathogen was grown in pure culture in small glass vials attached to strips of cardboard which were then buried vertically and horizontally in a light sandy soil in the field. From one to three cubic centimeters of chloropicrin was then injected to various depths alongside the vertical strips, each horizontal strip being buried to the particular depth of injection. Three days after treatment the vials were dug and plantings of the inoculum aseptically made in nutrient agar medium. Although definite

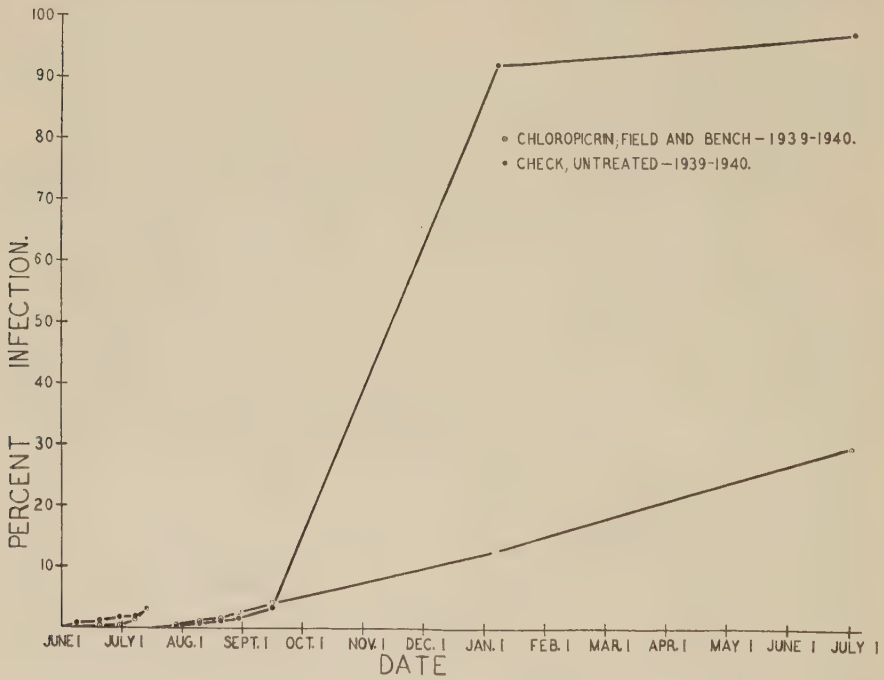

FIGURE 7. SEASONAL TREND OF FUSARIUM WILT INFECTION IN PLANTS OF THE VARIETY SOPHELIA GROWN IN UNTREATED INFESTED SOIL, COMPARED WITH INFECTION IN PLANTS IN SIMILAR SOIL TREATED WITH CHLOROPICRIN

June 1 to July 15, 1939 — in the field; July 15, 1939, to July 1, 1940 — in the greenhouse.

conclusions cannot be drawn from these preliminary tests in regard to required dosages and proper spacing of injections, it was readily shown that the pathogen is killed by the limited diffusion of chloropicrin vapor in all directions from the point of injection. For example, in one case the injection of two cubic centimeters to a depth of six inches killed the pathogen three inches above and below, and six inches laterally from, this point; three cubic centimeters injected to a depth of nine inches resulted in killing it at points from three to fifteen inches vertically and horizontally to nine inches.

The results of the above experiments clearly illustrate the efficacy of chloropicrin and formaldehyde in the control of Fusarium wilt. In the 1938 experiment, treatment of the bench soil alone with chloropicrin produced results comparable to those obtained by treatment of both the field and bench soil. The appearance of a comparatively large amount of infection in the

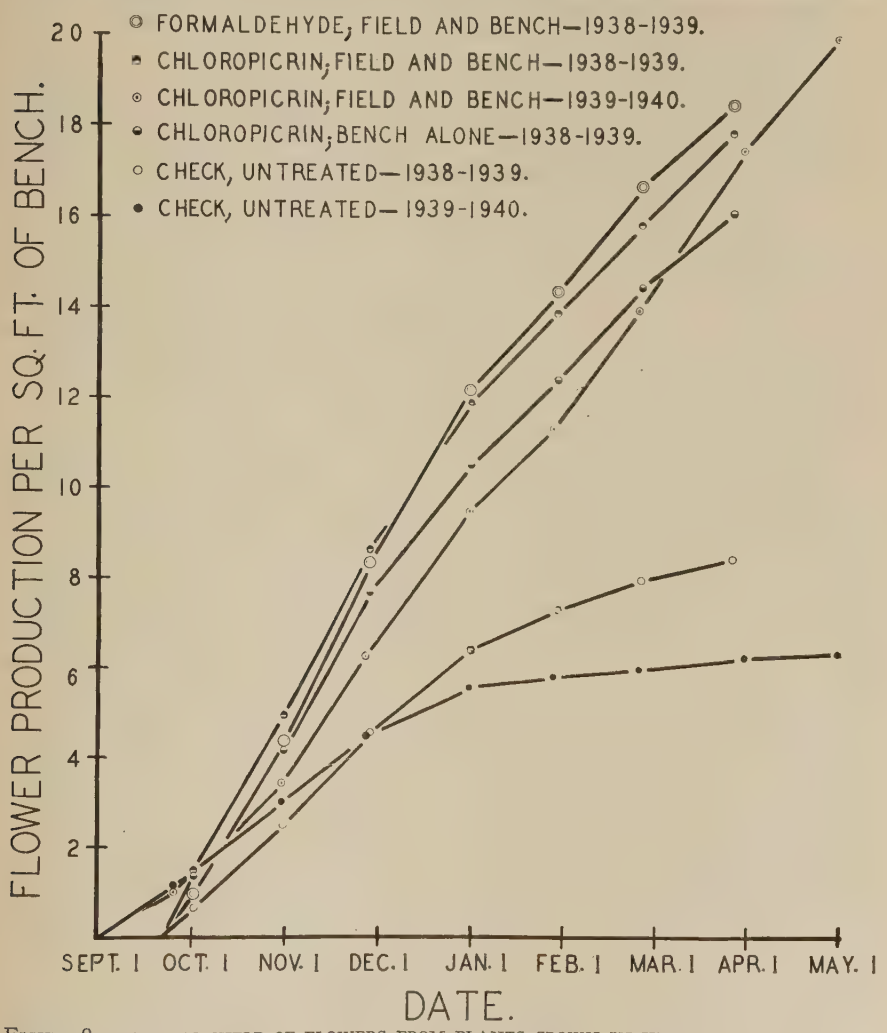

FIGURE 8. SEASONAL YIELD OF FLOWERS FROM PLANTS GROWN IN UNTREATED SOIL NATURALLY INFESTED WITH *F. Dianthi,* COMPARED WITH THE YIELD FROM PLANTS IN SIMILAR SOIL TREATED WITH CHLOROPICRIN OR FORMALDEHYDE

treated field plots in both experiments indicates that complete disinfestation of the field soil was not obtained. But, because all the soil available to a particular grower may be infested with *F. Dianthi,* and because infected plants and infested soil are often carried from one location to another, a

factorial design experiment was conducted in 1940 to determine when, in the seasonal history of carnations, treatment with chloropicrin is necessary for the best control.

In this experiment plants of the variety Sophelia were grown throughout the season in soil known to be infested with the pathogen. They were grown (a) before transplanting to the field in soil treated or not treated with chloropicrin, (b) in the field in similarly treated and untreated soil, and (c) in treated and untreated soil in the greenhouse benches. All combinations of these factors result in eight treatments. In January, 1940, approximately 2000 rooted cuttings were transplanted to untreated bench soil. An additional 2000 of the same age were similarly transplanted to flats of the same soil which had been previously treated with adequate amounts of chloropicrin. The following April, four contiguous beds in the field were each divided into eight equal plots. The eight compound treatments were then randomized in each bed, giving four replications of each treatment. The sixteen field-treated plots, together with the surrounding paths, were treated April 29 by injecting two-cubic-centimeter quantities in holes six inches in depth. The injections were staggered 10.5 inches apart in rows spaced nine inches apart, according to the recommendations of Taylor (29). This resulted in the application of approximately three cubic centimeters per square foot. A light rain which wet the soil surface shortly after treatment served as a temporary seal for the gas.

All field plots were planted May 16, each with approximately 115 plants. Toward the end of June two benches of old soil in the greenhouse were each divided into eight equal plots. The four replications of each treatment were subsequently transplanted to the greenhouse benches according to a randomized scheme by lumping the plants from two field replications. On July 3 chloropicrin was applied to a depth of three to four inches in the eight bench-treated plots, four in each bench, with the same rate and distribution as used in the field. After application, a water seal was maintained on the surface for three days.

Each plot was planted July 12 with approximately 182 plants. Flower-production records were taken on these plots from October 19 to December 9, 1940, and calculations made from these figures of the average yield per square foot of bench (table 6).

These data show that treatment of the bench soil with chloropicrin gave a highly significant increase in flower production even though the plants had previously been grown in infested soil. This is in agreement with the results of experiments described above. On the other hand, both the early treatment of greenhouse soil for the young plants and the treatment of the field soil were apparently ineffective in reducing the severity of Fusarium wilt, the differences in flower production being insignificant.

In the apparent absence of an effective method of treating field soil in the spring with chloropicrin, the use of new or well rotated field soil is suggested. To determine the feasibility of this method in combination with soil disinfestation of new and old field and bench soil, a factorial design experiment was conducted in 1940 using the very susceptible variety My Love. Plants were grown in (a) old infested field soil, and (b) new virgin field soil, treated and not treated with chloropicrin in each case. Later these were transplanted to (I) old infested bench soil, and (II) new virgin bench

TABLE 6. THE EFFECT OF TREATING GREENHOUSE AND FIELD SOIL WITH CHLOROPICRIN ON THE CONTROL OF FUSARIUM WILT IN THE VARIETY SOPHELIA, AS MEASURED BY FLOWER PRODUCTION, 1940

	Flower production per square foot of bench October 19 to December 9, 1940				
	Field soil treated		Field soil not treated		Mean†
	Bench soil treated	Bench soil not treated	Bench soil treated	Bench soil not treated	
Greenhouse soil for young plants treated	4.34	1.91	4.74	1.88	3.22
Greenhouse soil for young plants not treated	3.17	1.94	4.05	2.03	2.80
Mean*	3.75	1.92	4.40	1.95	
Mean†	2.84		3.17		

*Difference necessary for odds of 19:1 = 1.06 flowers.
Difference necessary for odds of 99:1 = 1.54 flowers.
†Difference necessary for odds of 19:1 = 0.75 flowers.
Difference necessary for odds of 99:1 = 1.09 flowers.

soil, treated and not treated with chloropicrin in each case. All combinations of these factors result in sixteen total treatments.

In April, 1940, four contiguous beds on old land and four on new land in the field were each divided into eight plots. This arrangement resulted in four replications of each of the eight old land plots and the eight new land plots, which were randomized. The treated plots, together with the surrounding paths, were treated April 30 to May 1 by injecting two-cubic-centimeter quantities in holes six inches in depth. The injections were staggered 10.5 inches apart in rows spaced nine inches apart, resulting in the application of approximately three cubic centimeters per square foot (29). Light rains which wet the soil surface shortly after treatment served as a temporary seal for the gas. All plots were planted May 17–18 at the rate of 60 plants per plot.

At the end of June, two greenhouse benches were each divided into 16 equal plots. The four replications of each treatment in the field were later planted in these plots according to a randomized scheme by lumping the plants in two field-replications. The old infested bench soil was left in half these plots. In the other half the old soil was removed, the bench-boards were disinfested by washing with a 1–25 aqueous formaldehyde solution, and the plots filled with new soil. Chloropicrin was applied to the bench-treated plots at the same rate and with the same distribution as used in the field, injections being made to a depth of three to four inches. A light water seal was maintained on the soil surface for about three days after treatment.

Throughout the summer counts were made, in the field, of the number of plants affected with Fusarium wilt in each plot. On July 19 all plants, except those completely killed, in two replications of a given treatment were combined and planted in the respective bench plots. Final counts of the number of plants which presumably became infected in the field were taken in the greenhouse August 20 (table 4).

By the end of August it was very apparent in the above experiment that plants grown in old field soil were much inferior to those grown in new field soil (figure 6). This effect is partly due to the greater amount of

Alternaria-blight infection which developed in the old land plots, but is largely due to a highly significant difference in the amount of Fusarium wilt in the two locations (table 4). It is also evident that field treatment with chloropicrin caused a considerable reduction in the disease on old land, the mean difference being significant with odds greater than 99:1. Even in the treated old-field plots, however, there was considerably more infection than in either the untreated or treated new-field plots at the end of August. Subsequently, the additional effect of old and new bench soil began to appear when infections developed in plants grown in untreated old bench soil, whereas few or none developed in those grown in treated old or new bench soil, or untreated new bench soil.

Discussion of Results

The experiments described above show that disinfestation of infested bench soil with formaldehyde, and particularly chloropicrin, results in a marked reduction in Fusarium-wilt infection and a consequent increase in flower production of susceptible varieties. This occurred even when known sources of inoculum in the form of infested field soil and scattered infected plants were introduced into these plots. Presumably, therefore, re-infestation of the treated bench soil occurs at a very slow rate. The severity of the disease could be further reduced, however, by eliminating such sources of re-infestation. Effective disinfestation of field soil was in most cases not usually obtained in the above experiments. This was apparently due, in the case of chloropicrin, to the low temperatures and high moisture content of the field soil in the spring when the treatment was applied. It may be possible to surmount this difficulty by fall treatment of infested field soil. In view of present evidence, however, the use of non-infested new or well rotated field soil is recommended.

In the control of Fusarium wilt it appears that the practice of changing the old soil annually can be eliminated by treating the old soil *in situ* with chloropicrin. Although growers have questioned the ability of old disinfested bench soil to produce carnation crops equal in vigor to those produced by new soil, observations made in the present investigation indicate otherwise. Weinard and Decker (31) presented evidence to substantiate these observations in the case of carnations grown in old soils disinfested with steam. The crops cut from these plants were as good as or better than crops from plants grown in new soils.

At the maximum dosage of chloropicrin used in these experiments, slightly more than three cubic centimeters per square foot, the total cost of material and labor for bench treatment is approximately one cent per square foot. On Long Island this is appreciably less than the labor cost of changing the bench soil. Furthermore, as shown in table 5, treatment of old infested bench soil may increase flower production from plants grown in such soil to the extent of 8 to 13 flowers per square foot. Consequently, at an average price of 3 cents apiece, the approximate net profit may vary from (24–1=) 23 cents to (39–1=) 38 cents per square foot of bench.

SUMMARY

Fusarium wilt, caused by *Fusarium Dianthi*, Prill. et Del., is a distinct and serious disease on Long Island.

The results of inoculations of various commercial varieties of carnations have shown marked differences in susceptibility to the disease.

The symptoms of Fusarium wilt are typically unilateral and involve yellowing, wilting, stunting, and necrosis. They are associated with the gradual upward extension of the pathogen and the discoloration of the vascular tissue of roots and stem. One shoot after another becomes affected until an entire plant is killed.

The signs of the disease are the clusters of sporodochia and pionnotes, the minute sclerotial bodies, and the white mycelium of the pathogen, which develop on the surface of affected tissue.

Various isolates of the pathogen have been identified as *Fusarium Dianthi* Prill. et Del. and their pathogenicity has been definitely established.

Probably the main source of the pathogen is infested soil in the greenhouse and field. The results of overwintering experiments show that *F. Dianthi* is able to survive two Long Island winters in the soil. Other important sources of inoculum are infected cuttings and plants.

The principal infection court for the pathogen is the roots. Infection also occurs, however, through wounds in the base of the main stem. At 70–80° F. the latent period of infection ranged from 12–28 days in the case of stem-wound inoculations to 23–45 days with root inoculations. At 50–65° F. this period lengthened to 41 days in the case of stem-wound inoculations, and 56–75 days in the case of root inoculations. On entering the roots or stem, the pathogen at first extends upward in the xylem, but gradually grows laterally into the pith on one side and the cambium, phloem, and cortex on the other side. With the exception of the vessels and tracheids, these tissues are usually completely disorganized by the ramifying hyphae.

The optimum, minimum, and maximum temperatures for the growth of the pathogen in culture are approximately 80°, 43°, and 97° F. respectively. The results of various inoculation tests show that high temperatures in the neighborhood of 75–80° F. increase the amount of infection in root inoculations, shorten the incubation period, and increase the rate of killing of infected plants; whereas, lower temperatures have the reverse effects. It is considered impossible to apply this information to the control of the disease in commercial greenhouses.

In controlling the disease the selection of healthy cuttings and the use of resistant varieties are recommended. The amount of Fusarium wilt on susceptible varieties is decreased by growing plants in new field and bench soil. In addition, on susceptible varieties grown in infested soil, the severity of the disease is appreciably decreased and flower production increased by soil disinfestation with either formaldehyde or chloropicrin. Since the results from field treatment with these materials were on the whole unsuccessful, it is concluded from these experiments that susceptible varieties should be grown in new or well rotated soil in the field, although the required length of rotation is unknown. Furthermore, new or well rotated soil should be used in the greenhouse benches or the old soil disinfested with chloropicrin or formaldehyde.

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